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Joint Asian Pacific Association of Gastroenterology (APAGE)–Asian Pacific Society of Digestive Endoscopy (APSDE) clinical practice guidelines on the use of non-invasive biomarkers for diagnosis of colorectal neoplasia

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ABSTRACT

Screening for colorectal cancer (CRC) is effective in reducing CRC related mortality. Current screening methods include endoscopy based and biomarker based approaches. This guideline is a joint official statement of the Asian Pacific Association of Gastroenterology (APAGE) and the Asian Pacific Society of Digestive Endoscopy (APSDE), developed in response to the increasing use of, and accumulating supportive evidence for the role of, non-invasive biomarkers for the diagnosis of CRC and its precursor lesions. A systematic review of 678 publications and a two stage Delphi consensus process involving 16 clinicians in various disciplines was undertaken to develop 32 evidence based and expert opinion based recommendations for the use of faecal immunochemical tests, faecal based tumour biomarkers or microbial biomarkers, and blood based tumour biomarkers for the detection of CRC and adenoma. Comprehensive up-to-date guidance is provided on indications, patient selection and strengths and limitations of each screening tool. Future research to inform clinical applications are discussed alongside objective measurement of research priorities. This joint APAGE–APSDE practice guideline is intended to provide an up-to-date guide to assist clinicians worldwide in utilising non-invasive biomarkers for CRC screening; it has particular salience for clinicians in the Asia-Pacific region.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer, with more than 1.9 million new cases and 1 million deaths reported worldwide in 2020.[1] The incidence and mortality of CRC are projected to increase in the next few decades, leading to a significant public health burden.[2] In particular, this rapid rise of CRC is most prominent in countries in the Asia-Pacific region.[3] A recent global analysis of the incidence and mortality of CRC in 36 countries showed that Asia-Pacific is one of the geographical regions that showed significant increases in new CRC cases. According to the projection from GLOBOCAN 2020, the total number of new CRC cases will rise from 1.03 million in 2020 to 1.76 million in 2040, with a substantial increase observed in Asian countries.[4] In addition, the global level of CRC screening has been growing, using a range of technologies. A recent evaluation found that the global expenditure incurred from CRC screening was estimated to be US$662.8 million in 2020 with a projected rise to US$728.6 million in 2026.[5] With rising disease incidence and cancer awareness, the demand for CRC screening is predicted to further escalate, and the Asia-Pacific region is expected to have the largest market growth for CRC screening.[5]

Currently, common screening tests for CRC include faecal occult blood tests (FOBTs), faecal immunochemical tests (FITs), flexible sigmoidoscopy and colonoscopy.[6 7] However, novel non-invasive tests, including tumour DNA and microbial markers, have recently become one of the major driving forces for a growth in expenditure on CRC screening.[8] The market size of these non-invasive tests is likely to be more than US$1000 million in 2027, with a compounded annual growth rate of more than 5.9%.[9]

Most population based programmes worldwide have adopted FITs and colonoscopies as their primary screening tests,[10 11] and studies have supported their efficacy in reducing CRC mortality and their cost effectiveness.[12 13] Despite improvement in the performance of FITs over guaiac based FOBTs, FITs have modest sensitivity in CRC detection (~0.8),[14 15] especially of early stage cancers, and low sensitivity for the detection of advanced colorectal neoplasia (ACN) (<0.5), with a high false negative rate.[16–18] In addition, adherence to FIT based screening programmes represents a major challenge, and one of the barriers to participation relates to the perception of the low accuracy of FIT.[19 20]

Colonoscopy has been recommended as a key screening tool based on guidelines from western

countries, in some cases as a first line screening tool.[21] However, an updated Asia-Pacific consensus has not recommended colonoscopy for first line screening in resource limited countries.[22] Although colonoscopy is considered to be the gold standard test, it is invasive, labour intensive, expensive, requires bowel preparation and needs a high level of expertise.[23 24] Furthermore, it has been shown that a large proportion of screening participants are reluctant to undergo colonoscopy due to fear and various perceptual factors.[25]

The incidence and mortality rates of CRC have shown a steady decrease in western countries, which is believed to be driven by changes in risk factors, early detection of cancer through screening and removal of precancerous polyps with colonoscopy.[26] The use of non-invasive screening tests that have the potential to detect both CRC and its precursor lesions may further allow risk stratification of appropriate patients for colonoscopy and potentially improve compliance, leading to a further reduction in CRC-related mortality.[27]

Recently, the American Gastroenterological Association (AGA) published an expert opinion based clinical practice update on the use of non-invasive CRC screening tools.[28] However, this practice update may have limited applicability in populations outside of North America whereby healthcare systems, screening uptake rates and availability of colonoscopy screening services may differ. Table 1 compares the existing non-invasive screening tests for CRC. While there is a growing demand for more accurate and affordable non-invasive CRC screening tests, there is a lack of guidance on their use in clinical practice.

This document aims to provide evidence based guidance on the use of non-invasive biomarkers for CRC screening. This practice guideline is a joint official statement of the Asian Pacific Association of Gastroenterology (APAGE) and the Asian Pacific Society of Digestive Endoscopy (APSDE). We discussed general principles for using the biomarkers, strengths, limitations, clinical applications and future development of non-invasive biomarkers. The experts primarily comprised specialists and primary care professionals involved in the provision of CRC screening; the guidance is primarily aimed at healthcare professionals who provide CRC screening in their clinical practice.

METHODOLOGY

Guideline development group

In February 2022, a steering committee representing APAGE and APSDE (FKLC, SCN, MCSW, KW and RARA) was established to develop the practice guideline statements. The steering committee invited members to form a joint task force to participate in the process of finalising guideline development. The selection criteria of the task force members were based on expertise in CRC through publication/research and participation as key members in national or regional guidelines. The guideline development group (GDG) included gastroenterologists, endoscopists, physician scientists, epidemiologists, primary care physicians and public health professionals, to ensure a wide range of expertise across all relevant disciplines. The GDG is aware that clinical decisions for individual patients may lead to deviations of practice from these guidelines. Hence this set of guidelines is not aimed at establishing a legal standard of care or as encouraging, and neither does it advocate or discourage the use of any particular test. The guideline development process included meetings, telephone conferences, online discussions and voting among members of the GDG between February 2022 and August 2022.

Evidence synthesis

The steering committee performed a systematic review of the literature according to the general principles proposed in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. We identified relevant studies published in the English language using AMED, BIOSIS Previews, EBM Reviews, Global Health, NASW Clinical Register, Embase, Ovid Medline and the Cochrane Trials Register in human subjects up to 25 November 2021, and subsequently to 1 February 2023, to check for additional literature (table 2). National and international guidelines on CRC screening were solicited. In addition, meeting abstracts from Digestive Disease Week, International Digestive Disease Forum, Asia Pacific Digestive Week, American College of Gastroenterology, AGA, American Society of Gastrointestinal Endoscopy, British Society of Gastroenterology, United European Gastroenterology Week and relevant published review articles from the preceding 5 years were screened. In addition to evidence from the search of electronic databases, evidence documented in existing guidelines which met the inclusion criteria was evaluated for inclusion in the document. All abstracts and articles were examined for relevance, with additional papers identified from cross checking of references and recommendations from the consensus group panel.

After eligible studies were identified, we assessed methodological quality of studies included, based on the Quality Assessment of Diagnostic Accuracy Studies, where applicable. Data were retrieved into a data extraction form by one reviewer and checked independently by a second reviewer to shortlist articles of relevance. The first draft statements were edited and revised by a core steering committee (FKLC, SCN, MCSW, KW and RARA) and were sent to each faculty member who agreed to participate via the voting. The recommendations were selected to cover general principles on use of non-invasive biomarkers,

Table 1 Comparison of clinically available non-invasive screening tests for colorectal cancer

Screening tool	Sample	Detection target	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Sensitivity to advanced adenoma (%) (95% CI)	Cost (US$)
gFOBT	Faeces	Haemoglobin	39 (25 to 55)[107]	94% (91 to 98)[107]	–	Euro$1.36-2.04 [108]
FIT**‡	Faeces	Haemoglobin	82 (63-92)[16]	93 (91-95)[16]	30 (25-34)[16]	Euro$ 3-4.5[108]
mt-sDNA (Cologuard)	Faeces	NDRG4 and BMP3 DNA methylation, KRAS mutations and haemoglobin	92.3 (DNA testing); 73.8 (FIT)[72]	89.8 (DNA testing); 96.4 (FIT)[72]	42.4 (DNA testing); 23.8 (FIT)[72]	US$600[109]
mSEPT9 (Epi ProColon)	Plasma	SEPT9 DNA methylation	61.8 (53-69.9)[110]	89.6 (83-93.8)[110]	27.4 (18.7-37.6)[111]	US$192[112]
Bacterial biomarker LR4+FIT* (M3CRC)	Faeces	Microbial markers and haemoglobin	94†[27]	81†[27]	56.8†[27] (m3+FIT)	250†

*LR4 is a combination of *Fusobacterium nucleatum*, a Lachnoclostridium species m3, *Bacteroides clarus* and *Clostridium hathewayi*.
†Data on file.
‡Threshold at 20 microgram/gram or above
FIT, faecal immunochemical test; gFOBT, guaiac based faecal occult based test; mt-sDNA, multi-target stool DNA.

Table 2 Literature search process

	Searches (inception to 1 February 2023)	Results
1	Colorectal neoplasms/or colonic neoplasms/or rectal neoplasms/	216 237
2	(colorectal cancer or colorectal adenoma) title, abstract	104 818
3	Biomarkers, tumour/or DNA methylation/or carcinoembryonic antigen/or neoplasm proteins/or epigenomics/	312 575
4	(biomarker* or molecular marker* or cancer maker* or haemoglobin or carcinoembryonic antigen or stool or faecal or faeces or urine or methylation or hypomethylat* or hypermethylat* or epigenetic) title, abstract	826 506
5	"Systematic review"/or meta-analysis/or (systematic review or meta-analysis) pt or (systematic review or meta-analysis) title, abstract	336 895
6	Early detection of cancer/or predictive value of tests/or prognosis/or risk factors/	1 635 367
7	(screening or predictive or prediction or predicting or diagnos* or prognostic or prognosis) title, abstract	3 809 084
8	1 or 2	232 868
9	3 or 4	1 017 702
10	6 or 7	4 699 370
11	5 and 8 and 9 and 10	770
12	Animals/not humans/	5 053 862
13	11 not 12	769

pt, publication type.

FITs, blood based tumour biomarkers, stool based tumour biomarkers and stool based microbial biomarkers, and future directions on the use of non-invasive biomarkers. To develop the recommendations, information on study characteristics and methods, participant characteristics, screening tests and performance of the screening tests was extracted. A narrative summary of all studies was undertaken, including tabulation of relevant study information and a quality assessment of the evidence, from which a draft document was produced. Additional publications of relevance were considered at the discretion of the consensus group panel as far as they were aware of.

The steering committee reviewed shortlisted articles and reached consensus on references which were considered appropriate based on the following criteria: (i) properly conducted diagnostic studies, prospective cohort study or randomised controlled trials; (ii) data pertaining to the Asia-Pacific population; and (iii) the latest international and national guidelines on novel non-invasive tools for CRC screening. In addition, the GDG adopted systematic reviews and original articles that are primarily conducted among Asian populations to formulate recommendations in the key areas. For studies conducted in western countries, the GDG evaluated whether the study findings of the articles could be applied in Asia-Pacific countries to determine if they should be included in the Delphi consensus process.

Key practice guidelines questions

The practice guideline was divided into six key areas: (i) general principles on use of non-invasive biomarkers; (ii) recommendations for FITs; (iii) recommendations for blood based tumour biomarkers; (iv) recommendations for stool based tumour biomarkers; (v) recommendations for stool based microbial biomarkers; and (vi) future directions on use of non-invasive biomarkers. For each area, relevant statements were drafted by members of the steering committee. The statements focused on current practice and areas of controversy in the use of novel non-invasive CRC screening tools, with particular relevance to countries in the Asia-Pacific region. Drafted statements were circulated to the panel members. Members were invited to amend or edit any statement as deemed appropriate based on expert opinion and the literature.

Modified Delphi consensus

We adopted a standardised process to provide a methodological framework for the development of the guidelines.[29] A two stage modified Delphi process was used to develop the consensus from June 2022 to August 2022. Firstly, the GDG coordinated the process of inviting international experts to join a panel in the first online meeting, drafted the statements based on findings from the literature search and discussed the underlying evidence that supported the recommendations. The statements, narrative summary and supporting references for each guideline question were uploaded onto an online platform, which was used to facilitate the guideline development process. In the first round survey, an up-to-date literature overview was presented for each of the statements. The international experts were invited to review the draft statements and provide their votes and comments via an online platform. The GDG members reviewed all of the votes and comments, and revised the statements accordingly.

In the second online meeting, individual panel members were assigned to present an overview of the literature for each individual statement, before the discussion and voting process. An enriched expanded literature summary was provided for each statement, and panel members were asked to vote based on review of the literature on a Likert scale, anchored by 1–5 (1=accept completely, 2=accept with some reservation, 3=accept with major reservation, 4=reject with some reservation, and 5=reject completely). The panel members were encouraged to furnish further comments and cite other relevant references. All votes were anonymous. Agreement of a statement by at least 80% of the task force (accept completely or accept with some reservation) was defined a priori as consensus.

For statements for which a consensus could not be reached, the entire group discussed and modified the statements accordingly, followed by a second round of voting. If consensus was still not reached, the statement was modified for the last time, and a third and final vote was conducted. The consensus method did not force agreement. Each statement was subsequently graded to indicate the level of available evidence and the strength of recommendation by all members of the GDG (table 3). The

Table 3 Quality of evidence and classification of recommendations

Quality of evidence	
I	Evidence obtained from at least one randomised controlled trial
II-1	Evidence obtained from well designed controlled trials without randomisation
II-2	Evidence obtained from well designed cohort or case-control study
II-3	Evidence obtained from comparison between time and places, with or without intervention
III	Opinion of respected authorities, based on clinical experience and expert committees
Classification of recommendations	
A	There is good evidence to support the statement
B	There is fair evidence to support the statement
C	There is poor evidence to support the statement but recommendation made on other grounds
D	There is fair evidence to refute the statement
E	There is good evidence to refute the statement

final document on each topic was written by the panel chair in conjunction with their working party. Consensus guideline statements displayed were followed by comments on the evidence and opinions. Statements are intended to be read in the context of the qualifying comments and not read in isolation. The final text was circulated and approved by participants. The consensus was finally endorsed by APAGE and APSDE.

RESULTS

We found 216 237 citations for review, resulting in 769 eligible articles (table 2). The recommendations of the practice guidelines were divided into five key areas and 32 statements, including target population for consideration of CRC screening using non-invasive biomarkers (statements 1–2), general recommendations on the use of non-invasive biomarkers (statements 3–9), faecal immunochemical tests (statements 10–13), blood based tumour biomarkers (statements 14–20), stool based tumour biomarkers (statements 21–26), stool based microbial biomarkers (statements 27–30) and future directions on the use of non-invasive biomarkers (statements 31–32). New knowledge generated from these statements and their strengths are shown in box 1.

Box 1 New knowledge

⇒ Faecal immunochemical test (FIT) is not recommended as a screening modality for advanced or non-advanced adenoma. Its use in participants with iron deficiency anaemia or acute diarrhoea should be avoided.
⇒ Plasma microRNA (miRNA) and stool based tumour markers, such as syndecan-2 and secreted frizzled related protein 2 (SFRP2) have potential to be used as primary colorectal cancer (CRC) screening tests, as they have high sensitivity and specificity to detect CRC.
⇒ The use of biomarker panels confers a higher discriminatory performance than a single biomarker. Combined use of stool based and blood based tumour biomarker tests could increase the sensitivity for CRC diagnosis.
⇒ Stool based microbial markers are sensitive to detect both CRC and adenomatous polyps. Microbial panel, such as a combination of *Fusobacterium nucleatum*, Lachnoclostridium gene marker (m3) and *Clostridium hathewayi,* has the potential to screen for advanced colorectal neoplasia and detect recurrent advanced colorectal neoplasia after polypectomy
⇒ Manipulation of gut microbiome has the potential to influence CRC risk.

Strengths and limitations

⇒ These guidelines are the first to provide evidence based, up-to-date guidance on the use of various non-invasive biomarkers for the diagnosis of colorectal neoplasia in Asia-Pacific regions and beyond.
⇒ Based on a comprehensive literature review, a guideline development group consisting of various experts in relevant disciplines participated in a two stage Delphi consensus process. Recommendations on the indications, patient selection and strengths and limitations of different non-invasive biomarkers, together with research priorities to inform future clinical application, are presented.
⇒ We anticipate that the application of these guidelines in clinical practice could optimise the yield of non-invasive biomarkers in detecting colorectal neoplasia, and allow an informed choice of screening modalities.
⇒ Some statements of recommendations were formulated based on a small number of studies with limited sample size. We suggest researchers should perform larger scale studies with translational potential to inform future updates on the summary recommendations presented in this guideline.

EXECUTIVE SUMMARY OF KEY RECOMMENDATIONS

Target screening population

- Target population for consideration of non-invasive biomarkers includes individuals of average risk for advanced colorectal neoplasia, identified by risk scores, who wish to know their risk for adenomas and CRC.
- Target population for consideration of non-invasive biomarkers includes individuals of high risk for advanced colorectal neoplasia who are reluctant to receive colonoscopy.

General recommendations for use of non-invasive biomarkers

- Non-invasive CRC screening programmes can only be successful if participants with positive tests understand the importance of, and are willing to undergo, timely high quality diagnostic colonoscopy, and those with negative tests undergo follow-up screening at appropriate intervals.
- The quality of screening programmes requires establishment of objective metrics, continuous monitoring of programme compliance and responsiveness to investigate and alter operations to achieve the highest adherence for better patient outcomes.
- Key performance indicators of non-invasive biomarker tests include participant adherence, punctual reporting of test results, prompt scheduling and completion of follow-up colonoscopy in participants with a positive test, and fastidious systems to ensure compliance to appropriate intervals for future CRC screening.
- Non-invasive CRC screening programmes should assess the appropriateness of participant selection for non-invasive testing.
- Once the screening test is completed, it is important that participants receive the test results in a timely fashion. We suggest that outreach is attempted in 100% of participants within 2 weeks of the test result.
- Participants with negative results should be informed of the appropriate recall interval for future screening and how recall will be made. All participants with a positive result should receive a colonoscopy.
- At least 80% of these patients should be offered a date for colonoscopy within 3 months and 100% within 6 months. A critical requirement for optimal patient outcomes is completion of colonoscopy.

Summary recommendations for use of faecal immunochemical tests

- FIT is the primary test of choice for population based CRC screening programmes in resource-limited countries.
- The use of FIT in screening for advanced or non-advanced adenomas is not recommended as it has limited diagnostic accuracy.
- One should practise caution in interpretation of FIT in male participants, individuals with a family history of CRC, smokers and users of aspirin or non-steroidal anti-inflammatory agents. We do not recommend the use of FIT in patients with iron deficiency anaemia

- FIT should be avoided during an episode of acute diarrhoea.

Summary recommendations for use of blood based tumour biomarkers

- Current data on methylated septin 9 for the detection of CRC/adenoma are based on simulation modelling, and larger scale studies are needed to support its clinical use.
- Plasma microRNA (miRNA) has potential to be used as a primary CRC screening test.
- There is a lack of adequate evidence supporting the use of plasma protein biomarkers for CRC screening.
- Circulating tumour DNA has the potential to determine the prognosis of CRC.
- The use of a biomarker panel has a higher discriminatory performance for CRC than a single biomarker.
- There is a necessity to explore the cost effectiveness of blood based tumour biomarkers in detecting colorectal adenoma and early stage CRC in future studies.
- Combined use of stool based and blood based tumour biomarker tests could increase the sensitivity for CRC diagnosis.

Summary recommendations for use of stool based tumour biomarkers

- Stool based tumour biomarkers are potential non-invasive screening tests for CRC.
- Evaluation of the upper gastrointestinal tract is not indicated for screening asymptomatic participants with positive multi-target stool DNA (mt-sDNA) and negative colonoscopy as the risk ratio of incident aerodigestive cancer is not higher than those with negative mt-sDNA and negative colonoscopy.
- Clinicians should recognise non-compliance with stool based biomarkers over time as one of the major barriers of screening uptake.
- The use of stool based tumour biomarkers to detect adenomatous polyps is not recommended.
- Similar to blood based tumour biomarkers, the use of a biomarker panel could offer higher discriminatory capability than a single biomarker for CRC screening.
- Further studies should be performed to explore the cost effectiveness of stool based tumour biomarkers in detecting colorectal adenoma and early stage CRC.

Summary recommendations for the use of stool based microbial biomarkers

- Stool based microbial biomarkers are sensitive to detect CRC and are superior to tumour based biomarkers for adenomatous polyps.
- Persistent non-adherence to stool based microbial biomarkers might represent a challenge in screening programmes.
- Microbial panel, such as a combination of *Fusobacterium nucleatum*, Lachnoclostridium gene marker (m3) and *Clostridium hathewayi*, has potential to screen for advanced colorectal neoplasia.
- Microbial panel, such as combination of *Fusobacterium nucleatum*, Lachnoclostridium gene marker (m3) and *Clostridium hathewayi*, has potential to detect recurrent advanced colorectal neoplasia after polypectomy.

Future directions on use of non-invasive biomarkers

- Manipulation of the gut microbiome has the potential to influence CRC risk.
- Stool based microbial markers have the potential to predict immunotherapy and chemotherapy responses in patients with CRC.

Target screening population

Statement 1. Target population for consideration of non-invasive biomarkers includes individuals of average risk for advanced colorectal neoplasia, identified by risk scores, who wish to know their risk for adenomas and CRC

(Level of agreement: A=46.7%, B=46.7%, C=0%, D=0%, E=6.7%.) (Quality of evidence: III; classification of recommendation: C)

Statement 2. Target population for consideration of non-invasive biomarkers includes individuals of high risk for advanced colorectal neoplasia who are reluctant to receive colonoscopy

(Level of agreement: A=66.7%, B=20.0%, C=6.7%, D=0%, E=6.7%.) (Quality of evidence: III; classification of recommendation: C.)

In asymptomatic average risk individuals of any age, CRC risk varies based on several factors, including genetics, gender, ethnicity, lifestyle and diet. Non-invasive modalities for CRC detection should be considered if the prevalence of risk factors and advanced colorectal neoplasia (ACN) is low/average whereas colonoscopy is preferred in participants with a higher risk for ACN. Simple clinical scoring systems have been developed to help stratify risk for ACN and have potential for tailoring CRC screening in average risk individuals.[30 31] Other risk algorithms, including the Asia-Pacific Colorectal Screening score[32] or its modified version which incorporated body mass index,[33] have been devised and validated to predict risk of ACN and are frequently used to risk stratify participants for optimal screening modalities. Individuals with average or moderate risk have been recommended to receive FIT as the primary screening test while those identified as high risk should be advised colonoscopy.[32] Programmes with higher uptake rates for FIT have greater clinical benefits and screening efficacy, with greater reductions in CRC related mortality. It is also more cost effective when participation rates are high.[34–36] Hence, for community based CRC screening programme to be effective, a substantial proportion of the population should be involved.[34]

However, a major reason for reduced participation rates in CRC screening programmes is perceived low sensitivity of FIT for detection of adenomas, whereby participants identify a potential threat of precancerous pathology being missed by FIT.[20] Hence average risk individuals who remained concerned of risks of adenomas might default screening if physicians provide FIT as the only screening option. In this context, non-invasive biomarkers that also detect colorectal adenomas may serve as an alternative and appealing option for these average risk individuals to increase screening uptake. As CRC incidence increases with age and is still relatively low at ages 45–49 years, the use of sensitive non-invasive tests in younger participants could result in substantial life years gained, while colonoscopy might be reserved for patients as they enter into higher CRC risk groups and are more susceptible to harbour advanced adenomas and CRC.[37]

The ideal screening programme would be one that is tailored to an individual's CRC risk. Although participants identified as above average risk are recommended to receive colonoscopy,[32 38] a large CRC screening programme consisting of Chinese participants found that several barriers had significantly limited colonoscopy uptake among high risk individuals. These

included financial difficulty (86.0%), limited service accessibility (58.2%), screening induced bodily discomfort (55.2%), physical harm (44.4%), embarrassment (40.1%), apprehension (38.8%) and time constraints (13.9%).[25] It is therefore anticipated that a significant number of high risk individuals would decline to have colonoscopy as a primary screening test in Asia. The advent of non-invasive biomarkers can potentially fill an unmet need in providing accurate risk prediction for both CRC and adenomas, thus enabling above average risk individuals to consider subsequent workup based on findings from non-invasive screening, especially in those who are reluctant to undergo colonoscopy.

Other guiding principles for the use of non-invasive biomarkers have been covered in the guidelines from the AGA institute,[28] which recommend the use of precision medicine to identify suitable individuals within the average risk cohort who may benefit from non-invasive screening by incorporating clinical genomic and lifestyle factors. Although strong evidence showed that CRC risk has increased in those aged 45–49 years, and screening from the age of 45 years is justified, CRC risk remains relatively low in this group of screenees and non-invasive biomarkers may be more appropriate for younger individuals.[28]

General recommendations on use of non-invasive biomarkers

Statement 3. Non-invasive CRC screening programmes can only be successful if participants with positive tests understand the importance of, and are willing to undergo, timely high quality diagnostic colonoscopy, and those with negative tests undergo follow-up screening at appropriate intervals.

(Level of agreement: A=93.3%, B=6.7%, C=0%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 4. The quality of screening programmes requires establishment of objective metrics, continuous monitoring of programme compliance and responsiveness to investigate and alter operations to achieve the highest adherence for better patient outcomes.

(Level of agreement: A=93.3%, B=6.7%, C=0%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 5. Key performance indicators of non-invasive biomarker tests include participant adherence, punctual reporting of test results, prompt scheduling and completion of follow-up colonoscopy in participants with a positive test, and fastidious systems to ensure compliance to appropriate intervals for future CRC screening.

(Level of agreement: A=73.3%, B=20.0%, C=6.7%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 6. Non-invasive CRC screening programmes should assess the appropriateness of participant selection for non-invasive testing.

(Level of agreement: A=73.3%, B=20.0%, C=6.7%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 7. Once the screening test is completed, it is important that participants receive the test results in a timely fashion. We suggest that outreach is attempted in 100% of participants within 2 weeks of the test result.

(Level of agreement: A=60.0%, B=33.3%, C=6.7%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 8. Participants with negative results should be informed of the appropriate recall interval for future screening and how recall will be made. All participants with a positive result should receive a colonoscopy.

(Level of agreement: A=86.7%, B=13.3%, C=0%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 9. At least 80% of these patients should be offered a date for colonoscopy within 3 months and 100% within 6 months. A critical requirement for optimal patient outcomes is completion of colonoscopy.

(Level of agreement: A=73.3%, B=20.0%, C=13.3%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

It is important for non-invasive CRC screening programmes to assess the suitability of patient selection for non-invasive tests. Individuals at high risk of CRC should preferably be screened with colonoscopy whereas those of low or average risk can be considered for non-invasive tests. A high quality screening programme should document and report efforts to obtain and record an accurate, three generation family cancer history with attention to explore the risk of CRC and advanced adenomas, including age at diagnosis, body mass index and smoking history, etc, which are known to impact CRC risk. Evidence suggests that adherence rate to CRC screening was generally more important than which strategy was used,[39] and several measures of adherence are required. If targets are not achieved, the underlying causes, including potential system/programme issues or factors relating to patient non-compliance, should be determined. It has been demonstrated that limiting the recommendation for CRC screening to colonoscopy can result in a lower completion rate for CRC screening compared with providing a choice between non-invasive tests or colonoscopy, especially among ethnic/racial minorities.[40] Significant differences in adherence to competing CRC screening tests between racial/ethnic groups has also been reported.[40]

A non-invasive CRC screening test is not complete until a follow-up colonoscopy is performed after a positive test. Hence a critical requirement for optimal patient outcomes is completion of colonoscopy. A study using data from a national screening programme in Taiwan examined the CRC death rates among more than 59 300 participants with a positive FIT. A 1.64-fold (95% CI 1.32 to 2.04) increased risk for CRC death was found in participants who did not receive colonoscopy compared with participants who received colonoscopy, after adjustment for differences in baseline characteristics.[41] Another study using the Taiwanese screening programme database compared the risks of any CRC and advanced stage CRC among FIT positive participants with different timing of follow-up colonoscopies.[42] Compared with colonoscopy within 1–3 months, risks were significantly higher when colonoscopy was delayed by more than 6 months for any CRC (adjusted OR (aOR) 1.31, 95% CI 1.04 to 1.64) and advanced stage disease (aOR 2.09, 95% CI 1.43 to 3.06). The risks continuously increased when colonoscopy was delayed by more than 12 months for any CRC (aOR 2.17, 95% CI 1.44 to 3.26) and advanced stage disease (aOR 2.84, 95% CI 1.43 to 5.64). There were no significant differences for colonoscopy follow-up at 3–6 months for risk of any CRC or advanced stage disease.

There are many reasons patients fail to undergo a colonoscopy follow-up. These include health issues of higher priority that may not permit a colonoscopy to be safely performed,[43] patients who refuse the colonoscopy follow-up, anxiety or fear of the procedure and lack of awareness of the importance. A survey conducted in an Asian population based on the Health Belief Model evaluated the factors associated with willingness to

participate in confirmatory colonoscopy follow-up after a positive FIT.[44] It was found that higher perceived threat (aOR 1.62, 95% CI 1.31 to 2.01), higher cues for action (aOR 2.18, 95% CI 1.68 to 1.82), lower perceived barriers (aOR 0.42, 95% CI 0.34 to 0.42) and higher health behaviour scores (aOR 1.30, 95% CI 1.05 to 1.60) were associated with participation in confirmatory colonoscopy.

Non-adherence to colonoscopy follow-up has also been demonstrated to be driven by system barriers which may include failure to arrange the colonoscopy, failure to inform the patient of a positive test results, lack of action by the colorectal surgical or gastroenterology clinic staff after the colonoscopy and failure to contact the patient.[45] Given that delays in colonoscopy of 6 months or longer after a positive FIT have been shown to be associated with higher risks of advanced adenomas, CRC and advanced stage CRC, programmes should measure the proportion of follow-up colonoscopies recommended and aim for ≥95% to be performed within 6 months of a positive non-invasive test. All patients with positive tests should be recommended for colonoscopy and at least 80% should be offered a date for colonoscopy within 3 months and 100% within 6 months. CRC system level navigation programmes that track test positive patients and contact patients by telephone to schedule appointments may increase adherence to colonoscopy follow-up.[28] It is proposed that outreach is attempted in 100% of patients within ≤2 weeks of the test results.[28] Furthermore, patients with negative results should be informed of the suitable recall interval for future tests.

Summary of recommendations for use of faecal immunochemical tests

Statement 10. FIT is the primary test of choice for population based CRC screening programmes in resource limited countries.

(Level of agreement: A=80%, B=20%, C=0%, D=0%, E=0%.) (Quality of evidence: I; classification of recommendation: A.)

Statement 11. The use of FIT in screening for advanced or non-advanced adenomas is not recommended as it has limited diagnostic accuracy.

(Level of agreement: A=46.7%, B=33.3%, C=13.3%, D=0%, E=6.7%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 12. One should practise caution in interpretation of FIT in male participants, individuals with a family history of CRC, smokers and users of aspirin or non-steroidal anti-inflammatory agents.

(Level of agreement: A=53.3%, B=33.3%, C=6.7%, D=0%, E=6.7%.) (Quality of evidence: II-2; classification of recommendation: B.)

Statement 13(a). We do not recommend the use of FIT in patients with iron deficiency anaemia.

Statement 13(b). FIT should be avoided during an episode of acute diarrhoea.

(Level of agreement: A=66.7%, B=20%, C=6.7%, D=0%, E=6.7%.) (Quality of evidence: II-2; classification of recommendation: B.)

Commonly used stool tests include gFOBT and FIT. Randomised controlled trials have shown that annual or biennial gFOBT reduces CRC mortality[46] and were more cost effective compared with no screening,[47] with reasonably high sensitivity and specificity. It is also one of the most affordable screening tests among all screening modalities, including flexible sigmoidoscopy, faecal DNA and colonoscopy, hence making it a feasible choice for resource limited countries with limited endoscopic capacity. Among underserved patients whose CRC screening was not up to date, mailed outreach invitations resulted in markedly higher CRC screening compared with usual care. Outreach was more effective with FIT than with colonoscopy invitation.[48]

FIT also has some limitations. Although the performance of the FIT has been improved and is now widely used in Europe for CRC screening, its use remains limited in the detection of early stage CRC. In addition, FIT is not sensitive for detecting adenoma[16]; the sensitivity of FITs for advanced adenoma varied between 25% and 40%, with modest positive and negative likelihood ratios.[16] FIT has no utility for serrated colorectal lesion detection. FIT has a relatively low specificity, leading to many false positive screens and hence has a significant cost implication.[49] Some factors have been found to be associated with false negative results, which included male sex (RR 1.83, 95% CI 1.53 to 2.19), family history of CRC (RR 1.61, 95% CI 1.19 to 2.15) and smoking (RR 1.93, 95% CI 1.52 to 2.45). The use of aspirin/non-steroidal anti-inflammatory agents could lead to false positive results (RR 1.16, 95% CI 1.06 to 1.27).[49] The use of FIT for CRC screening should be avoided in patients with iron deficiency anaemia.[50] A meta-analysis showed a sensitivity of 0.58 (95% CI 0.53 to 0.63) and a specificity of 0.84 (95% CI 0.75 to 0.89) in these patients. Sensitivities of FOBT for a positive stool culture in the two studies of patients with diarrhoea were 0.38 (95% CI 0.31 to 0.45) and 0.87 (95% CI 0.75 to 0.95), with specificities of 0.85 (95% CI 0.79 to 0.89) and 0.58 (95% CI 0.45 to 0.71).[50]

Summary recommendations for blood based biomarkers

Statement 14. Current data on methylated septin 9 for the detection of CRC/adenoma are based on simulation modelling, and larger scale studies are needed to support its clinical use.

(Level of agreement: A=58%, B=33%, C=8%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 15. Plasma microRNA (miRNA) has potential to be used as a primary CRC screening test.

(Level of agreement: A=33.3%, B=46.7%, C=13.3%, D=0%, E=6.7%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 16. There is a lack of adequate evidence supporting the use of plasma protein biomarkers for CRC screening.

(Level of agreement: A=73.3%, B=20.0%, C=6.7%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C).

Statement 17. Circulating tumour DNA has the potential to determine the prognosis of CRC.

(Level of agreement: A=46.7%, B=33.3%, C=6.7%, D=6.7%, E=6.7%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 18. The use of a plasma biomarker panel has a higher discriminatory performance for CRC detection than a single biomarker.

(Level of agreement: A=80%, B=13.3%, C=0%, D=6.7%, E=0%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 19. There is a necessity to explore the cost effectiveness of blood based tumour biomarkers in detecting colorectal adenoma and early stage CRC in future studies.

(Level of agreement: A=80%, B=20%, C=0%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Statement 20. Combined use of stool based and blood based tumour biomarkers could increase the sensitivity for CRC diagnosis.

(Level of agreement: A=60%, B=33.3%, C=0%, D=0%, E=6.7%.) (Quality of evidence: II-2; classification of recommendation: A.)

Blood based screening tests have been considered to be minimally invasive and require little patient preparation. Emerging evidence suggests novel circulating biomarkers as potential alternatives for CRC detection which mostly consist of blood methylation markers, circulating miRNA[52] and plasma protein biomarkers. Currently, only one blood based biomarker test, known as the septin 9 blood test or Epi proColon (Epigenomics), has been approved by the Food and Drug Administration (FDA) for CRC screening. It is a polymerase chain reaction (PCR) based qualitative test that detects methylation of the promoter region of septin 9 DNA. It is indicated for screening participants who have been offered but declined first line CRC screening tests, such as FIT, and diagnostic colonoscopy is still necessary after a positive Epi proColon result.

The accuracy of septin 9 in CRC diagnosis has been assessed in a subset of 7941 asymptomatic, average risk adults aged >50 years in the USA and Germany undergoing screening colonoscopy.[53] The test characteristics were based on two PCR replicates in 53 patients with CRC and 1457 patients without CRC. The sensitivity and specificity for CRC were 48.2% and 91.5%, respectively. The sensitivity for advanced adenoma detection was 11.2%. In a subanalysis using a third polymerase chain replicate in available samples, sensitivity and specificity for CRC were 63.9% and 88.4%, respectively.[53] Two meta-analyses of case-control studies of septin 9 tests using colonoscopy as the reference reported summary estimates of sensitivity and specificity for detection of CRC of 62–71% and 91–92%, respectively.[54 55]

A validated microsimulation screening analysis–colon model has been used to evaluate screening alternatives to colonoscopy every 10 years or annual FIT, including mt-sDNA every 1 or 3 years, CT colonography every 5 years, capsule endoscopy every 5 or 10 years and septin 9 every 1 or 2 years. Assuming perfect adherence, annual septin 9 resulted in more quality adjusted life years gained and CRC cases and deaths averted than annual FIT, but with high rates of colonoscopy. Currently, the septin 9 test is not recommended in the US Preventive Services Task Force or the US Multi-Society Task Force guidelines because there are concerns about its sensitivity and specificity.[37 46 56] Apart from simulation modelling data, there are also a lack of data showing morbidity or mortality benefit. Larger CRC screening studies using blood based tests are now underway.

Several plasma miRNAs have been studied as potential biomarkers for CRC detection.[57] Data analysis from 223 CRC patients and 130 healthy controls reported that the sensitivity of miR-24, miR-320a and miR-423–5 p for early stage CRC was 77.8%, 90.7% and 88.9%, respectively. In addition, the combination of a few miRNAs has been investigated for potential detection of early stage CRC, including a five plasma miRNA detection panel and a three plasma miRNA detection panel.[58–60] A meta-analysis of 35 studies showed that miRNAs (e.g. miRNA-16, cel-miRNA-39 and cel-miRNA-238) had a sensitivity of 0.80, specificity of 0.80 and AUC of 0.87,[61] whereas a single plasma miRNA (miR-139) had a sensitivity of 0.89, specificity of 0.91 and AUC of 0.96 for CRC detection.[61]

A systematic review and meta-analysis showed that certain miRNA markers (miR601, miR760 and miR29a) had high sensitivities for detecting precancer lesions. Receiver operating characteristic (ROC) curve analysis showed that plasma miR-601 and miR-760 also had diagnostic value for detection of advanced neoplasia.[62] In a recent systematic review of 34 studies comprising 3454 CRC cases and 2556 controls, 617 plasma miRNAs were reported to be dysregulated.[63] A panel of four miRNAs achieved the highest AUC of 0.943 with 83.3% sensitivity and 93.1% specificity. Sensitivity and specificity of 28 individual miRNAs in the diagnosis of CRC were both 76%, indicating good discriminative ability of miRNAs as biomarkers for CRC. Overall, higher specificity can be achieved by using a panel of biomarkers.[63] However, key limitations remain; some predictive miRNAs are not specific for one type of cancer and most of these studies have not yet been evaluated beyond the proof-of-principle and pilot stage. Also, not all miRNA markers were subsequently studied and validated by other groups. Furthermore, conventional detection methods for miRNAs are quantitative PCR (qPCR), microarray and next generation sequencing, but no one method is completely ideal for clinical application.

Protein markers represented the most common target in both the prospective cohort based studies and screening studies but are limited by low sensitivity and specificity for early lesions, and the discriminatory ability of these proteins, including AREG, LRG1 and MIC-1/GDF15, have been insufficient for clinical implementation.[64] In a meta-analysis of genome wide and proteome wide data from CRC tumours, a combination of four proteins (TRIM28, PLOD1, CEACAM5 and P4HA1) had 100% sensitivity and specificity, but thus far there are only few published studies. Their diagnostic potential needs confirmation in larger cohorts, and potential confounders, such as age, gender and ethnicity should be considered.[65]

An increasing number of studies are reporting on the potential use of circulating tumour DNA (ctDNA) in the management of patients with CRC. ctDNA offered an early marker of long term prognosis in non-resectable disease, with changes after one cycle of systemic therapy demonstrating prognostic value.[66] The presence of ctDNA in the blood is a result of biological processes, namely tumour cell apoptosis and/or necrosis, and can be used to monitor different cancers by targeting cancer specific mutation. A study involving 123 patients with locally advanced rectal cancer showed that total ctDNA at diagnosis was of modest prognostic value as patients with ctDNA levels above the 75th percentile had a higher risk of disease recurrence than those below it (HR 2.48, p=0.007).[67] A separate study of 159 patients with locally advanced rectal cancer showed that the presence of postoperative ctDNA was predictive of disease recurrence, irrespective of the use of adjuvant chemotherapy Furthermore, the findings of a study involving 47 patients with rectal cancer showed that recurrence free survival was shorter in patients with detectable ctDNA after completion of chemoradiotherapy.[68] A systematic review including data from nine studies and 615 patients demonstrated a correlation between ctDNA level and clinical outcomes of response to neoadjuvant therapy.[69] Future prospective studies will help promote the efficient development and integration of this technology into clinical care and prognosis of CRC.

This systematic review summarises the evidence from studies that used samples collected before the onset of symptoms and found that panels of biomarkers performed better than single markers. Potentially promising biomarkers included anti-p53 antibodies, proteins such as AREG, MIC-1/GDF15, LRG1 and FGF-21, metabolites and/or metabolite profiles, non-coding RNAs and DNA methylation, as well as repurposed routine laboratory tests, such as ferritin and the triglyceride–glucose index. Although some biomarkers are not accurate enough to be used alone, they showed consistently promising results as a marker for early diagnosis of CRC. They could serve as a supplement to methylated septin 9 testing in a future multi-marker panel.[64] Therefore, blood based and stool based tumour markers

may be combined to improve efficacy for CRC screening. Addition of faecal based tests improved the sensitivity of blood based tests, such as inclusion of FIT with SEPT9.

Whether the use of blood based tumour biomarkers is cost effective, compared with conventional screening methods, is unknown. A systematic review of 51 studies of blood markers for primary human CRC was performed.[70] The markers were divided into broadly four groups: nucleic acids (RNA/DNA/mRNA/miRNAs), cytokines, antibodies and proteins. The most promising circulating markers identified among the nucleic acids were NEAT_v2 non-coding RNA, SDC2 methylated DNA and SEPT9 methylated DNA. The most promising cytokine to detect CRC was interleukin 8, and the most promising circulating proteins were CA11-19 glycoprotein and DC-SIGN/DC-SIGNR. Sensitivities of these markers for detecting CRC ranged from 70% to 98% and specificities from 84% to 98.7%. It was found that the SEPT9 test was more cost effective than no screening but less cost effective than FIT. However, this comprehensive review did not identify enough numbers of cost effectiveness analysis of these blood based tumour biomarkers to support territory wide population based CRC screening. Hence the cost effectiveness of these biomarkers and their use in different regions remains speculative and should be further investigated.

Summary recommendations for use of stool based tumour biomarkers

Statement 21. Stool based tumour biomarkers are potential non-invasive screening tests for CRC.

(Level of agreement: A=50%, B=42%, C=8%, D=0%, E=0%.) (Quality of evidence: II-2; classification of recommendation: B.)

Statement 22. Evaluation of the upper gastrointestinal tract is not indicated for screening asymptomatic participants with positive multi-target stool DNA (mt-sDNA) and negative colonoscopy as the risk ratio of incident aerodigestive cancer is not higher than those with negative mt-sDNA and negative colonoscopy.

(Level of agreement: A=53.3%, B=40.0%, C=0%, D=6.7%, E=0%.) (Quality of evidence: II-2; classification of recommendation: B.)

Statement 23. Clinicians should recognise non-compliance with stool based biomarkers over time as one of the major barriers of screening uptake.

(Level of agreement: A=73.3%, B=20.0%, C=6.7%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: B.)

Statement 24. The use of stool based tumour markers to detect adenomatous polyps is not recommended.

(Level of agreement: A=73.3%, B=26.7%, C=0%, D=0%, E=0%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 25. Similar to blood based tumour biomarkers, the use of stool biomarker panels could offer higher discriminatory capability than a single biomarker for CRC screening.

(Level of agreement: A=86.7%, B=6.7%, C=0%, D=0%, E=6.7%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 26. Further studies should be performed to explore the cost effectiveness of stool based tumour biomarkers in detecting colorectal adenoma and early stage CRC.

(Level of agreement: A=93.3%, B=6.7%, C=0%, D=0%, E=0%.) (Quality of evidence: III; classification of recommendation: C.)

Faecal DNA markers have been recommended by some medical professional societies as a non-invasive strategy for CRC screening in average risk individuals.[71] Cross sectional studies have confirmed initial diagnostic performance in case-control studies of a mt-sDNA test, which was approved by the US FDA in 2014. mt-sDNA, known as Cologuard, includes an FIT, two DNA methylation markers (*BMP3* and *NDRG-4*), an assessment of *KRAS* mutations and a marker of total human DNA. mt-sDNA is approved only in average risk adults aged 45–85 years. In a pivotal trial of 9989 participants comparing mt-sDNA and FIT, sensitivity for detection of CRC and advanced adenoma plus sessile serrated polyps ≥10 mm was 92% and 42% with mt-sDNA and 74% and 24% with FIT ($p=0.002$ and $p<0.001$, respectively), but overall specificity for all lesions was lower (87% vs 95%).[72] Its low sensitivity for advanced adenoma renders this test unsuitable for screening of precancerous lesions. Because of the reduced specificity in the pivotal study, there will be some false positives. mt-sDNA sensitivity for CRC was shown not to differ between black and white participants in the USA.[73]

A subanalysis of aerodigestive cancers was performed in 13.3% of participants in the pivotal mt-sDNA trial who had a negative (normal or only non-advanced adenoma) colonoscopy and comprehensive cancer follow-up.[74] After a median of 5.4 years, incident aerodigestive cancers occurred in 2.4% of participants with discordant results (negative colonoscopy and positive mt-sDNA) and in 1.1% with concordant results (negative colonoscopy and negative mt-sDNA) with no difference in the risk ratio between the groups. When compared with the Surveillance, Epidemiology and End Results (SEER) data, aerodigestive cancer incidence was lower in the concordant group than expected by SEER (risk ratio 0.4, 95% CI 0.2 to 0.6) and not significantly greater in the discordant group than expected by SEER (risk ratio 0.8, 95% CI 0.3 to 1.9). The authors suggested that patients with a negative high quality colonoscopy should not undergo further testing. The US Multi-Society Task Force on Colorectal Cancer recommends that in the absence of symptoms or signs of upper gastrointestinal pathology, an evaluation of the upper gastrointestinal tract is not indicated.

Other stool based tumour biomarkers included SDC2, with reported sensitivity of 0.81 (95% CI 0.74 to 0.86), specificity of 0.95 (95% CI 0.93 to 0.96) and AUC of 0.96 (95% CI 0.94 to 0.97)[75]; methylation of multiple DNAs has a sensitivity of 0.71 (95% CI 0.69 to 0.73), specificity of 0.92 (95% CI 0.90 to 0.93) and AUC of 0.93 (95% CI 0.91 to 0.95), and for multiple markers, sensitivity is 0.76 (95% CI 0.71 to 0.80), specificity 0.88 (95% CI 0.84 to 0.91) and AUC of 0.91 in two respective studies.[76 77]

Stool based biomarker tests, like all other faecal screening modalities, have compliance issues because adherence over time decreases; the reasons for this are similar to those seen in FIT based screening. Typical stool based tumour biomarkers, such as SFRP2, and miR-92a and miR-21, when pooled together, had low accuracy to detect colorectal adenomas, with a sensitivity of 0.57 (95% CI 0.41 to 0.72) and specificity of 0.76 (95% CI 0.66 to 0.89). AUC was 0.75.[78] In a meta-analysis involving 29 articles (80 studies), 55 studies focused on single miRNA assays and the other 25 on multiple miRNA assays. Multiple miRNA assays showed better diagnostic accuracy compared with single miRNA assays. Thus it is important to test multiple useful miRNAs to increase the credibility of results in clinical examination.[79]

There is a scarcity of cost effectiveness evaluations for stool based tumour biomarkers when used in population based CRC screening programmes. The mt-sDNA test was associated with increased patient life years with every 3 year testing but at greater cost than other screening modalities. Significant increases in sensitivity and/or substantial decrease in cost are therefore necessary to make mt-sDNA screening cost effective relative to other modalities,

such as colonoscopy. Hence mt-sDNA performed annually was not a recommended strategy due to efficiency ratios being larger than the benchmark colonoscopy strategy.[80] There is a knowledge gap to compare the cost effectiveness of different types of stool based tumour biomarkers used in community based screening. In addition, these stool based biomarkers should be evaluated in different ethnicities and populations outside of North America.

Summary recommendations for use of stool based microbial biomarkers

Statement 27. Stool based microbial markers are sensitive to detect both CRC and adenomatous polyps.

(Level of agreement: A=35.7%, B=57.1%, C=7.1%, D=0%, E=0%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 28. Persistent non-adherence to stool based microbial biomarkers might represent a challenge in screening programmes.

(Level of agreement: A=71.4%, B=14.3%, C=7.1%, D=0%, E=7.1%.) (Quality of evidence: III; classification of recommendation: C).

Statement 29. Microbial panel such as a combination of *Fusobacterium nucleatum*, Lachnoclostridium gene marker (m3) and *Clostridium hathewayi* has potential to screen for advanced colorectal neoplasia.

(Level of agreement: A=67%, B=33%, C=0%, D=0%, E=0%.) (Quality of evidence: II-2; classification of recommendation: A.)

Statement 30. Microbial panel such as a combination of *Fusobacterium nucleatum*, Lachnoclostridium gene marker (m3) and *Clostridium hathewayi* has potential to detect recurrent advanced colorectal neoplasia after polypectomy.

(Level of agreement: A=58%, B=42%, C=0%, D=0%, E=0%.) (Quality of evidence: II-2; classification of recommendation: B.)

In recent years, the gut microbiome has been shown to be involved in CRC pathogenesis with increasing potential of utilising the microbiota as CRC biomarkers and the prospect for modulating the microbiota for CRC prevention or treatment.[89] Human studies have reported microbial composition and ecology changes in patients with CRC, and functional studies in animal models have indicated the roles of several bacteria in driving colorectal carcinogenesis, such as *Fusobacterium nucleatum* and certain strains of *Escherichia coli* and *Bacteroides fragilis*. These data provide new insights into harnessing the gut microbiota for clinical applications, such as gut microbiota analysis as screening, predictive or prognostic biomarkers.

In a meta-analysis assessing studies of stool based microbial markers in PubMed, Embase, Cochrane Library and Web of Science databases up to December 2017, data from 1198 participants (629 CRC and 569 healthy controls) from 10 controlled studies and seven articles reported the diagnostic performance of *Fusobacterium nucleatum* for CRC detection. They identified an area under the receiver operating characteristic curve (AUROC) of 0.86 (95% CI 0.83 to 0.89) with a pooled sensitivity of 0.81 (95% CI 0.64 to 0.91) and specificity of 0.77 (95% CI 0.59 to 0.89), suggesting the potential of using stool based microbial biomarkers in detecting CRC.[81]

In more recent studies, a new Lachnoclostridium gene marker (labelled as 'm3') was evaluated for the diagnosis of colorectal adenoma.[82] It is a species specific bacterial gene marker that is capitalised on machine learning algorithms and rich metagenome datasets. Based on metagenomics data from 589 Asian participants, the marker 'm3' was found to be significantly enriched in participants with CRC and adenoma. A recent analysis showed that faecal m3 levels were significantly higher in patients with adenoma than in control participants. Combination of m3 with FIT showed high sensitivity (87.6%, 56.8%) and specificity (78.1%, 78.1%) for CRC and advanced adenoma, respectively. It has also been shown that m3 performed better than other bacterial markers, such as *Fusobacterium nucleatum* and *Clostridium hathewayi*, in diagnosing colorectal adenomas. A separate study that recruited 676 participants (210 CRC, 115 advanced adenoma, 86 non-advanced adenomas and 265 non-neoplastic controls) examined faecal abundances of *Fusobacterium nucleatum*, a Lachnoclostridium species m3, *Bacteroides clarus* and *Clostridium hathewayi* by qPCR. Combining the scores of the four microbial markers (4Bac), 4Bac was found to be more sensitive for diagnosing CRC and advanced adenoma than FIT.[82] Although stool based microbial markers appear to have superior sensitivity than stool based tumour markers, head-to-head comparisons between these two tests are lacking.

For universal use, microbial markers should be robust across populations with different lifestyle and dietary patterns. Based on the combined analysis of 526 metagenomic samples from Chinese, Austrian, American, German and French cohorts, seven CRC enriched bacteria distinguished cases from controls with an AUC of 0.80 across different populations.[83] In addition, in a metagenomic profiling study of CRC faecal microbiomes to validate microbial biomarkers in ethnically different cohorts, 20 microbial gene markers that differentiated CRC and control microbiomes were identified, and four markers were validated in the Danish, French and Austrian cohorts. qPCR measurements of two of the genes accurately classified patients with CRC in the independent Chinese cohort with an AUC of 0.84and OR of 23.[84] These data suggest that stool microbial markers using PCR on multiple bacteria appear robust across different populations and geography.

Future microbiome biomarker development will likely include not only qPCR but digital PCR, 16s sequencing[85 86] or whole genome sequencing approaches, depending on the accuracy and cost of each platform. Large scale, prospective, multi-ethnic studies are needed to confirm their universal use for non-invasive CRC diagnosis. Application of direct shotgun metagenomics to diagnosis is not cost efficient due to cumbersome experimental procedure and heavy computing workload. Targeted detection of identified microbial marker candidates using qPCR on single or multiple bacteria species or gene markers is recommended for convenient clinical application.

Certain stool based biomarkers were found to have a high discriminatory capability to detect recurrent adenomas. In a recent study, individuals enrolled in a polyp surveillance study from 2009 to 2019 were recruited. These eligible individuals were found to have adenoma on index colonoscopy who underwent polypectomy and had regular surveillance colonoscopy according to international guidelines. The study included a total of 161 baseline and 104 follow-up samples. Among patients with adenoma recurrence, *Fusobacterium nucleatum* and m3 increased while *Clostridium hathewayi* were unchanged in follow-up versus baseline samples. Among patients without recurrence, *Fusobacterium nucleatum* and m3 were unchanged while *Clostridium hathewayi* decreased ($p<0.05$) in follow-up versus baseline samples. The AUROC for detecting recurrent adenoma was 0.95 (95% CI 0.84 to 0.99), with 90.0% sensitivity and 87.0% specificity for detecting recurrent adenoma. A combination of m3, *Fusobacterium nucleatum* and *Clostridium hathewayi* at follow-up sample achieved an AUROC of 0.74 (95% CI 0.65 to 0.82) with 81.3% sensitivity and 55.4% specificity for detecting recurrent adenoma.[83] Additional studies are needed to confirm its value in predicting adenoma recurrence.

In addition, one study compared the faecal microbiota of patients diagnosed with adenoma, advanced adenoma and carcinoma before and after treatment. After treatment, the microbiota of patients with

carcinoma changed significantly more than the other groups and closely resembled those of patients with a normal colon, suggesting that treatment for carcinoma was not only successful for removing the carcinoma but also at reducing the associated bacterial communities.[87] It may be possible to use microbiome based biomarkers to not only predict the presence of lesions but also to assess the risk of recurrence due to these changes in the microbiota. It should be noted that microbial panels using other bacteria, such as *Clostridium symbiosum*, *Parvimonas micra* and pks+*Escherichia*coli may also have potential to screen for and detect recurrence of colorectal neoplasia.[88]

For screening participants with an abnormal abundance of pathogenic microbes and negative colonoscopy, we suggest that physicians should offer advice on modification of lifestyle habits that could modulate gut microbiota. These include low animal protein intake, low fat intake and high fibre consumption; weight reduction; and administration of probiotics.[89] A systematic review of clinical trials showed that probiotic/synbiotic administration improved enteric microbiota by reducing the abundance of potentially harmful bacteria, such as *Fusobacterium*, *Porhyromonas*, *Pseudomonas* and *Enterococcus*.[90] They should also be reminded to adhere to the screening programme in subsequent surveillance.

Several limitations of stool based microbial markers should be addressed. Firstly, the level of evidence of our recommendations is II-2 or III, and the number of studies on stool based microbial biomarkers is limited. Hence we have highlighted the potential of these biomarkers for diagnosis of colorectal neoplasia, and more definitive recommendations on their use in clinical practice will need more support from data in future large scale studies. In addition, there exists significant interindividual variation in gut microbiome, influenced by factors such as age, sex, dietary habits, smoking, body mass index and antibiotic use.[90 91] Most studies did not match these factors between patients with colorectal neoplasia and control subjects in their analyses. The gut microbiome is dynamic and evolves with the development of the neoplasia. Hence there is significant heterogeneity in the diversity of the faecal microbiome between geographically distinct populations and across nations.[92 93]

Another possible limitation of these studies is the lack of standardisation in the collection and processing of the samples. The sample collection methods, DNA extraction kits used, analytic approaches, microbiome identification method, choice of primers as well as temperature and time until long term storage (which could influence microbiome composition) are highly heterogeneous between studies.[94 95] In addition, the use of microbial markers might be less affordable than other screening modalities, and they are not commonly available in certain Asia-Pacific regions. Future evaluations of the compliance rates of using stool based microbial biomarkers as screening tests should be performed. Furthermore, the surveillance interval of re-screening and their cost effectiveness in population based screening programmes should be examined.

Future directions on use of non-invasive biomarkers

Statement 31. Manipulation of gut microbiome has the potential to influence CRC risk

(Level of agreement: A=62%, B=31%, C=8%, D=0%, E=0%.) (Quality of evidence: I; classification of recommendation: B.)

Statement 32. Stool based microbial markers have the potential to predict immunotherapy and chemotherapy responses in patients with CRC.

(Level of agreement: A=92%, B=8%, C=0%, D=0%, E=0%.) (Quality of evidence: II-2; classification of recommendation: B.)

Emerging evidence suggests that microbiota modulation can be associated with reduction in CRC related bacteria and potentially CRC risk. In a randomised, double blind, placebo controlled trial involving patients who had colonic polypectomy, oral treatment with a synbiotic formula led to significant changes in gut microbiota, reduced colorectal proliferation and improve epithelial barrier function.[96] Studies have also shown that the gut microbiota plays an essential role in intestinal epigenomic mechanisms of the host.[97–99]

It has long been recognised that the gut microbiota can modify the pharmacokinetics of various drugs, including anticancer therapies, thereby influencing therapeutic outcomes and/or side effects following chemotherapy and immunotherapy. Irinotecan, a chemotherapeutic agent commonly used for the treatment of metastatic CRC, caused adverse effects that were largely influenced by bacterial β-glucuronidase. Metagenomic and metabolomic profiling of patients' gut microbiota could be informative before choosing this drug to predict side effects.[100]

Emerging preclinical and clinical studies reported that gut microbiota affects the efficacy of immunotherapy. Immune checkpoint therapies are often used in association with chemotherapies and can also be positively or negatively impacted by the gut microbiota in terms of toxicity and therapeutic effect, as shown for anti-CTLA-4, anti-PD-L1 and anti-PD-1 antibodies. At least three clinical trials have demonstrated that the gut microbiota can be used to help predict response to PD-1/PDL-1 immunotherapies in solid epithelial tumours.[101] It was found that in the gut microbiota of patients with non-small cell lung cancer and kidney cancer treated with PD-1 inhibitors, levels of *Akkermansia* in the stool of responders were significantly higher than those of non-responders.[101] In melanoma patients treated with PD-1 inhibitors, it was found that responders' stool were rich in *Faecalibacterium* and *Ruminococcus*, and non-responders were rich in *Bacteroides*. The gut microbiota of responders in patients with hepatocellular carcinoma receiving PD-1 inhibitor therapy was also higher in *Akkermansia* and *Ruminococcus*.[102] These preclinical and clinical studies support the role of gut microbiota in modulating the efficacy of immunotherapy for various cancers.

There are other novel non-invasive screening tests that have been evaluated in the recent decade with preliminary findings published, such as a breath test for volatile organic compounds,[103] urine volatilome and metabolom signatures,[104] and cell free DNA as part of multi-cancer early detection blood panels.[105] As evidence pertinent to these novel biomarkers is accumulating, the guideline development group will consider expanding the scope of recommendations on CRC screening tests in future guideline updates. During the panel discussion meetings, the members were of the view that data on non-invasive biomarkers were relatively preliminary, with some screening modalities being studied in a limited number of subjects. This precluded certain statements from being more prescriptive. Hence the Delphi process has been adopted to inform readers on the quality of evidence and the classification of recommendations. We suggest researchers should perform larger scale studies with translational potential to inform future updates on the summary recommendations presented in this guideline.

CONCLUSION

With mortality from CRC among the leading causes of cancer deaths worldwide, there is an urgent need to increase screening, and therefore detect early, less invasive stage of disease to improve survival. New CRC screening strategies using more precise, non-invasive tools have the potential to increase national screening uptake rates due to their non-invasive nature and convenience for patients. Box 2 shows the individuals who could benefit from non-invasive biomarkers

Box 2 Individuals who will benefits from non-invasive colorectal cancer screening and their choice

Who will benefit most from non-invasive colorectal cancer (CRC) screening?

Two groups of screening participants will benefit from these non-invasive biomarkers, including:

⇒ Average risk individuals for advanced colorectal neoplasia, as identified by risk scores, who wish to know their risk for both adenoma and CRC; and

⇒ High risk individuals for advanced colorectal neoplasia who are reluctant to receive colonoscopy

Who will benefit from other non-invasive tests instead of the faecal immunochemical test (FIT)?

The use of FIT in screening for advanced or non-advanced adenomas is not recommended as it has limited diagnostic accuracy. Furthermore, patients with iron deficiency anaemia and acute diarrhoea should avoid using FIT as this leads to low sensitivity. They may opt for other non-invasive tests, such as stool based microbial biomarkers, which have high discriminatory capability for detection of both adenomatous polyps and CRC.

How to choose among different non-invasive tests?

Non-invasive tests should be chosen based on their acceptability, diagnostic accuracy, cost effectiveness and preferences of screening participants. Certain non-invasive tests, such as microbial biomarkers, could be used for participants who are keen to know their risk of adenomatous polyps.

for diagnosis of CRC, and the rationale to choose among various screening tests.

When given a choice, most individuals with an average risk of colorectal cancer typically indicate that they would prefer a stool based screening test for colorectal cancer over colonoscopy. In addition, it has been shown that participants offered informed choice of different CRC screening tests were significantly more likely to be adherent to screening programme, irrespective of what screening test was selected (FIT or colonoscopy).[106] Quality metrics for non-invasive screening programmes should be developed and programme performance should be assessed periodically, and practice guidelines updated every 5–10 years. Systems should be instituted to achieve optimal programmatic screening adherence and ensure timely colonoscopy to complete the screening spectrum in patients with a positive non-invasive screening test.

The latest generation of stool DNA and microbial biomarker tests are a significant achievement based on knowledge of the pathogenesis of colorectal neoplasia coupled with recent advances in technology that allow detection of minute amounts of human DNA and microbial DNA assayed from faecal material. Increased sensitivity with future optimised and enhanced versions of these tests, particularly for detection of adenomas, would likely increase the clinical efficiency ratio for non-invasive biomarkers compared with other screening strategies. Clinical application in risk stratification and interval cancer screening (ie, between screening colonoscopies) may be implemented in the future. Expanded applications with improved sensitivity and specificity of future non-invasive biomarkers is promising and could involve surveillance of CRC and adenoma. These potential applications will need to be adequately tested in appropriate clinical trials. Ultimately, the most effective test is the one that is well accepted by the target population.

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